Physiology & Behavior 104 (2011) 702–708

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Changes in taste reactivity to intra-oral hypertonic NaCl after lateral parabrachial injections of an α_2-adrenergic receptor agonist

Carina A.F. Andrade [a,b,*], Glaucia M.F. Andrade-Franzé [a], Laurival A. De Luca Jr. [a], Alan Kim Johnson [c,d,e,f], José V. Menani [a]

[a] Dept of Physiology and Pathology, School of Dentistry, UNESP, Araraquara, SP, 14801-903, Brazil
[b] Dept of Physiology, Institute of Biomedical Sciences, Federal University of Alfenas, Unifal-MG, Alfenas, MG, 37130-000, Brazil
[c] Dept of Psychology, University of Iowa, Iowa City, Iowa 52242-1407, USA
[d] Dept of Pharmacology and Health, University of Iowa, Iowa City, Iowa 52242-1407, USA
[e] Dept of Human Physiology, University of Iowa, Iowa City, Iowa 52242-1407, USA
[f] Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242-1407, USA

ARTICLE INFO

Article history:
Received 21 March 2011
Received in revised form 13 July 2011
Accepted 14 July 2011

Keywords:
Palatability
Taste
Adrenoceptors
Thirst
Sodium appetite
Hindbrain

ABSTRACT

Bilateral injections of moxonidine, an α_2-adrenoceptor and imidazoline receptor agonist, into the lateral parabrachial nuclei (LPBN) enhance sodium appetite induced by extracellular dehydration. In the present study, we examined whether LPBN moxonidine treatments change taste reactivity to hypertonic NaCl solution administered into the mouth by intra-oral (IO) cannula. Male Holtzman rats prepared with IO and bilateral LPBN cannulas received subcutaneous injections of furosemide (FURO; 10 mg/kg) and captopril (CAP; 5 mg/kg) to induce hypovolemia with mild hypotension and an accompanying salt appetite and thirst before testing the taste reactivity to oral infusions of 0.3 M NaCl (1.0 ml/min). In the first experiment 45 min after subcutaneous injections of FURO + CAP or vehicle, moxonidine was bilaterally injected into the LPBN, and then 15 min later both bodily and oral–facial ingestive and rejection responses to 0.3 M NaCl delivered through the IO cannula were assessed. Both LPBN vehicle and moxonidine treated rats showed increased ingestive and decreased rejection responses to the IO hypertonic solution. The IO 0.3 M NaCl infusion-evoked ingestive and rejection taste related behaviors were comparable in the LPBN vehicle- vs. the LPBN moxonidine-injected groups. In a second experiment, rats received the same FURO + CAP treatments and LPBN injections. However, beginning 15 min after the LPBN injections, they were given access to water and 0.3 M NaCl and were allowed to consume the fluids for most of the next 60 min with the free access intake being interrupted only for a few minutes at 15, 30 and 60 min after the fluids became available. During each of these three brief periods, a taste reactivity test was conducted. On the three taste reactivity tests rats that received LPBN vehicle injections showed progressive declines in ingestive responses and gradual increases in rejection responses. However, in contrast to the LPBN vehicle treated rats, animals receiving bilateral injections of LPBN moxonidine maintained a high number of ingestive responses and a low number of rejection responses throughout the test period even in spite of evidencing substantial water and 0.3 M NaCl consumption during the periods of free access. The results suggest that after α_2-adrenoceptor agonist delivery to the LPBN the acceptance of 0.3 M NaCl is sustained and the negative attributes of the solution are minimized. The maintained positive rewarding qualities of 0.3 M NaCl are likely to account for why LPBN moxonidine treated rats show such a remarkable salt appetite when assayed by the volume of hypertonic 0.3 M NaCl consumed.

1. Introduction

Sodium chloride (NaCl) is an important constituent of the extracellular fluid compartment and the major determinant of plasma osmolality and extracellular fluid volume. The acquisition and ingestion of water and of salty substances are necessary behavioral responses for an animal to recover from deficits in body fluids. Sodium deficiency results in behaviors collectively known as sodium appetite (a.k.a. salt appetite) and which can be operationally defined by an

* Corresponding author at: Department of Physiology, Institute of Biomedical Sciences, Federal University of Alfenas, Unifal-MG, 37130-000, Alfenas, MG, Brazil. Tel./fax: +55 16 3301 6488.
E-mail addresses: carina@unifal-mg.edu.br, carina_andrade@yahoo.com (C.A.F. Andrade).

0031-9384
doi:10.1016/j.physbeh.2011.07.020

increased ingestion of sodium solutions of concentrations which are normally avoided [1–3].

In the hindbrain, important inhibitory mechanisms for the control of water and NaCl intake have been demonstrated in the lateral parabrachial nucleus (LPBN) [4–10]. The LPBN, a pontine structure that lies dorsolateral to the superior cerebellar peduncle, is reciprocally connected to forebrain areas that have been implicated in the maintenance of blood pressure and body fluid homeostasis, such as the paraventricular nucleus of the hypothalamus, the central nucleus of the amygdala and the median preoptic nucleus. The LPBN is also richly interconnected with medullary regions, which include the area postrema (AP) and the medial portion of the nucleus of the solitary tract (mNTS), [11–18]. Cells in the LPBN are activated after ingestion of sodium solutions by dehydrated rats or in rats that received intragastric loads of hypertonic NaCl [19–21], suggesting that the LPBN might receive inhibitory visceral or taste signals. Therefore, the LPBN may integrate and relay taste and visceral signals that ascend from AP/mNTS en route to forebrain areas involved in the control of fluid and electrolyte balance [7–9,22,23].

The inhibitory mechanisms of the LPBN are modulated by different neurotransmitters like serotonin, cholecystokinin, glutamate, corticotrophin releasing factor, opioids and noradrenaline [7–10,24–32]. Activation of α_2-adrenoceptors with bilateral LPBN injections of moxonidine (α_2-adrenoceptor/imidazoline receptor agonist) or noradrenaline strongly enhances 0.3 M NaCl intake induced by subcutaneous treatment with the diuretic furosemide (FURO) when combined with a low dose of the antihypertensive drug, captopril (CAP) [24,32,33]. This suggests that activation of α_2-adrenoceptors in the LPBN may reduce the effects of inhibitory mechanisms that limit sodium intake [24,32,33]. The effects of α_2-adrenoceptor agonist treatment of the LPBN on sodium intake are not due to a non-specific facilitation of all ingestive behaviors, because sucrose solution intake is not affected by bilateral LPBN injections of moxonidine [33].

A taste reactivity test determining the frequency of ingestive and rejection behavioral reactions or fixed action patterns in response to intra-orally delivered solutions was originally developed by Grill and Norgren[34]. This method assesses the occurrences of species-typical affective behavioral reactions [such as ingestive-related tongue protrusions or negative (rejection) gapes] in response to oral stimulation [34,35]. Lesions placed within either the NTS, parabrachial nucleus (PBN), or the parvocellular ventral posteromedial thalamic nucleus (VPMpc) disrupt the shift in taste reactivity observed in intact animals after sodium deficiency [36]. Lesions placed in the NTS and PBN, but not the VPMpc, also block increases in home-cage intake observed in intact, sodium deficient rats [36].

Since α_2-adrenoceptor activation with the administration of moxonidine into the LPBN greatly increases NaCl intake in free access intake tests, the present studies tested whether LPBN α_2-adrenoceptor stimulation modifies taste reactivity responses to 0.3 M NaCl in rats with an experimentally-induced sodium appetite. The results of the experiments indicate that before animals ingest 0.3 M NaCl and water, LPBN moxonidine treatment does not increase or decrease the number of ingestive or rejection behaviors in comparison to those seen in LPBN vehicle treated rats. However, the findings do demonstrate that unlike LPBN vehicle treated animals showing decreased ingestive and increased rejection responses over the course of restoring body sodium and water, rats receiving LPBN moxonidine maintain a high level ingestive responses and a low number of rejection responses throughout a period of fluid repletion.

2. Material and methods

2.1. Animals

Male Holtzman rats weighing 290 to 310 g were housed in individual stainless steel cages with free access to normal sodium (0.5–1.0%) diet (Guabi Rat Chow, Paulínia, SP, Brazil), water and 0.3 M NaCl solution. Temperature was maintained at 23 ± 2 °C, and humidity was maintained at 55 ± 10% on a 12:12 light–dark cycle with light onset at 7:30 AM. The Ethical Committee for Animal Care and Use from the Dentistry School of Araraquara — UNESP approved the experimental protocols used in the present study (protocol 06/2006). The experimental protocols also followed the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 80-23, 1996).

2.2. Cerebral and IO cannulas

Rats were anesthetized with ketamine (80 mg/kg of body weight) combined with xylazine (7 mg/kg of body weight) and placed in a Kopf stereotaxic instrument. The skull was leveled between bregma and lambda. Stainless steel 23-gauge cannulas were implanted bilaterally above the LPBN using the following coordinates: 9.4 mm caudal to bregma, 2.1 mm lateral to the midline, and 4.2 mm below the dura mater, according to Paxinos and Watson [37]. The tips of the cannulas were positioned at a point 2 mm above each LPBN. The cannulas were fixed to the cranium using dental acrylic resin and jeweler screws, and 20-gauge metal obturators were used to fill the cannulas between tests. Immediately after the implantation of LPBN cannulas, all animals were also implanted with chronic IO cannulas. Each oral cannula (heat-flared PE 50 tubing) entered the mouth just lateral to the first maxillary molar. The tubing was tunneled subcutaneously to ascend lateral to the skull, and posterior to the nape of neck where the free end was exteriorized. The IO cannulas do not interfere with the normal eating behavior of the animal and allow the direct infusion of solutions into the mouth. The rats were allowed to recover for 6 days before drug injections were made into the LPBN.

2.3. Injections into the LPBN

Bilateral injections into the LPBN were made using 5-μl Hamilton syringes connected by polyethylene tubing (PE-10) to 30-gauge injection cannulas. At time of testing, obturators were removed and the injection needle (2 mm longer than the guide cannulas) was introduced in the brain. All the injections into the LPBN were 0.2 μl for each site and performed over a period of 1 min, with 1 additional min allowed to elapse before the injection needle was removed from the guide cannula to avoid reflux. The movement of an air bubble inside the polyethylene tubing connected to the syringe confirmed drug flow. The obturators were replaced after injection, and the rats were placed back into the cage.

2.4. Drugs

Moxonidine hydrochloride (0.5 nmol/0.2 μl) (Solvay Pharma, Hannover, Germany) dissolved in a mix of propylene glycol and water 2:1 (vehicle) was injected into the LPBN. Vehicle was injected as control.

The natriuretic/diuretic drug FURO (10 mg/ml; Sigma Chem., St Louis, MO, USA) was dissolved in alkaline saline (0.9% NaCl, pH was adjusted to 9.0 with NaOH) and administered s.c. at the dose of 10 mg/kg of body weight. The angiotensin converting enzyme inhibitor CAP (5 mg/ml; Sigma Chem., St. Louis, MO, USA) was dissolved in saline (0.9% NaCl) and administered s.c. at the dose of 5 mg/kg of body weight. The pH 9.0 saline solution was used as the vehicle control for FURO and normal 0.9% saline as the vehicle control for CAP.

2.5. Taste reactivity test

Prior to the testing period, rats with LPBN and IO cannulas were each given a 3-day habituation period during which they were exposed to the taste reactivity chamber for 10 min, followed by a 1 ml

infusion of water. Testing began 6 days after surgery, with a 48-hour interval between tests.

2.5.1. Experiment 1 — taste reactivity to 0.3 M NaCl in rats treated with sc FURO + CAP

On each test day, each rat was treated with sc FURO (10 mg/kg of body weight) + CAP (5 mg/kg of body weight) or vehicle + saline. At this time, water, food and 0.3 M NaCl were removed from the rat's home cage. Forty-five minutes later, rats received bilateral injections of moxonidine (0.5 nmol/0.2 μl) or vehicle (0.2 μl) into the LPBN. Immediately after the LPBN injections, the rat's oral cannula was connected to a stimulus delivery tube, consisting of PE 50 tubing attached to an infusion pump. The rats were then placed in a cylindrical plexiglas test chamber (30 cm diameter). Fifteen minutes later (i.e., 1 h after FURO + CAP sc and 15 min after LPBN injections), 0.3 M NaCl was infused into the mouth of the animal at a constant rate (1.0 ml/min) for 1 min. The rats were given four tests. For each test the rats were divided into two groups and each group received one of the following treatments: 1) vehicle + saline (sc) + vehicle (LPBN), 2) vehicle + saline (sc) + moxonidine (LPBN), 3) FURO + CAP (sc) + vehicle (LPBN), and 4) FURO + CAP (sc) + moxonidine (LPBN). The sequence of the treatments in each group was balanced, and by the end of experiments, all animals had received all four treatments. During testing, rats had no access to water, 0.3 M NaCl and food.

2.5.2. Experiment 2 — taste reactivity to 0.3 M NaCl in rats treated with sc FURO + CAP combined with LPBN moxonidine injections after free access to water and 0.3 M NaCl

The results from experiment 1 (see below) indicated that LPBN moxonidine treatment did not enhance ingestive responses or decrease rejection responses to IO 0.3 M NaCl in comparison to LPBN vehicle treated rats 1 h after both groups had received FURO + CAP. Consequently, a second experiment was conducted to determine if taste reactivity remained unaltered in LPBN vehicle treated vs. LPBN moxonidine treated rats even after periods of 0.3 M NaCl and water consumption.

On each test day, each rat received FURO (10 mg/kg of body weight) + CAP (5 mg/kg of body weight) treatment sc and had water, 0.3 M NaCl and food removed from the home cage. Forty-five minutes later, rats received bilateral injections of moxonidine (0.5 nmol/0.2 μl) or vehicle (0.2 μl) into the LPBN. Fifteen minutes after the LPBN injections, burettes (0.1 ml subdivisions) with water and 0.3 M NaCl were available in the rat's home cage, and the rats were allowed to ingest both fluids continuously for 60 min, except for a few minutes at 15, 30 and 60 min after being given free access to both fluids. During each of these three brief periods, the rats were given a taste reactivity test. During each test the rat was removed from the home cage and placed in a taste reactivity chamber. The IO cannula of the rat was connected to a stimulus delivery tube, consisting of PE 50 tubing attached to an infusion pump and 0.3 M NaCl was infused into the mouth of the animal at a constant rate (1.0 ml/min) for 1 min, while the behavior was videotaped. At the end of each taste reactivity test, until all three tests were completed, the rat was placed back into its home cage, where water and 0.3 M NaCl were available, and the rat was allowed to drink until the next taste reactivity test. Water and 0.3 M NaCl ingested was also measured at 15, 30 and 60 min after starting the access to these fluids.

The rats received 2 tests. In each test, all rats received FURO + CAP (sc) and were then divided into two groups. One group received LPBN vehicle injections and the other LPBN moxonidine injections. The order of the tests was counterbalanced so that all animals received all treatments.

2.5.3. Video recording and analysis of taste reactivity

For all protocols described above, the behavior of each rat was videotaped during testing via a mirror mounted beneath the transparent floor of the test chamber. The recorded image was enlarged so the face and mouth of the rat filled the entire screen.

The behavior of each rat was scored for the occurrence of ingestive, rejection (aversive), and "neutral" taste reactivity components (see [35] for a description and discussion of taste reactivity analysis components and classification). Ingestive actions were characterized by *paw licking*; *lateral tongue protrusions*, non-rhythmic protrusions past the lip followed by forward extension; and *tongue protrusions*, rhythmic tongue protrusions along the midline. Neutral components were rhythmic *mouth movements* at the same or lower frequency as rhythmic tongue protrusions; and *passive dripping*, the passive leaking of fluid from the mouth. Rejection behaviors were *gapes*, large openings of the mandible and retraction of the lower lips; *chin rubbing*, bringing the mouth in direct contact with the floor and projecting the body forward; *face washing*, either a single wipe over the face with the paws or a bout of several wipes; *forelimb flails*, shaking of the forelimb; *head shaking*; *paw treading*, planting of the limbs on the floor and alternating forceful strikes forward and backward; and rapid *locomotion* around the chamber. This taste reactivity analysis components and classification has been previously used [53,54].

The behaviors were counted each time they occurred as a single event and were considered as discrete events in accordance with previous studies [36,55]. Videotapes were scored in a slow motion analysis at l/30 to 1/l0 normal speed. The means for ingestive, rejection or neutral score were computed for each group. The ingestive and rejection scores were independently analyzed, since they represent phenomenological different categories of behavior.

2.6. Histology

At the end of the tests, the animals received bilateral injections of 2% Evans blue solution (0.2 μl) into the LPBN. They were deeply anesthetized with sodium thiopental (80 mg/kg) and perfused transcardially with 0.9% NaCl followed by 10% formalin. The brains were removed, fixed in 10% formalin, frozen, cut in 50-μm sections, stained with cresyl violet, and analyzed by light microscopy to confirm the injection sites in the LPBN.

2.7. Statistical analysis

The results are reported as means ± SEM. One-way analysis of variance (ANOVA) or two-way repeated-measures ANOVA (using treatment and time as factors), followed by Student Newman Keuls tests were used for comparisons of the results from experiments 1 and 2, respectively. Differences were considered significant at $P<0.05$.

3. Results

3.1. Experiment 1 — taste reactivity to 0.3 M NaCl by rats treated with sc FURO + CAP that received moxonidine injections into the LPBN

The FURO + CAP treatment combined with vehicle injected into the LPBN increased ingestive [$F(3,28)=4.5$; $p<0.05$] and decreased rejection behaviors [$F(3,28)=8.2$; $p<0.05$] to IO 0.3 M NaCl when compared to control treatment (vehicle + saline sc combined with vehicle into the LPBN) (Fig. 1). Bilateral injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN did not change these responses in rats with no access to water and 0.3 M NaCl (Fig. 1).

3.2. Experiment 2 — taste reactivity to 0.3 M NaCl by FURO + CAP treated rats that received LPBN moxonidine injections and had free access to water and 0.3 M NaCl

As previously demonstrated, bilateral injections of moxonidine (0.5 nmol/0.2 μl) into the LPBN increased FURO + CAP-induced 0.3 M

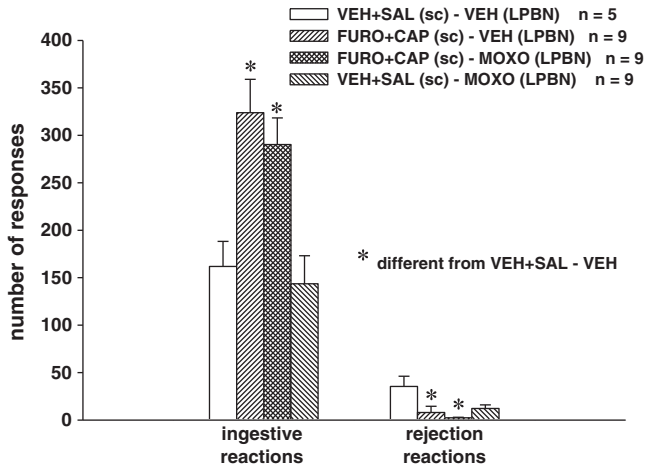

Fig. 1. Ingestive and rejection behavioral responses to intra-oral (IO) infusion of 0.3 M NaCl in rats treated with sc furosemide + captopril (FURO + CAP) or sc vehicle + saline (VEH + SAL) combined with bilateral injections of vehicle (VEH) or moxonidine (MOXO, 0.5 nmol/0.2 μl) into the LPBN. Results expressed as means ± SEM. n = number of animals.

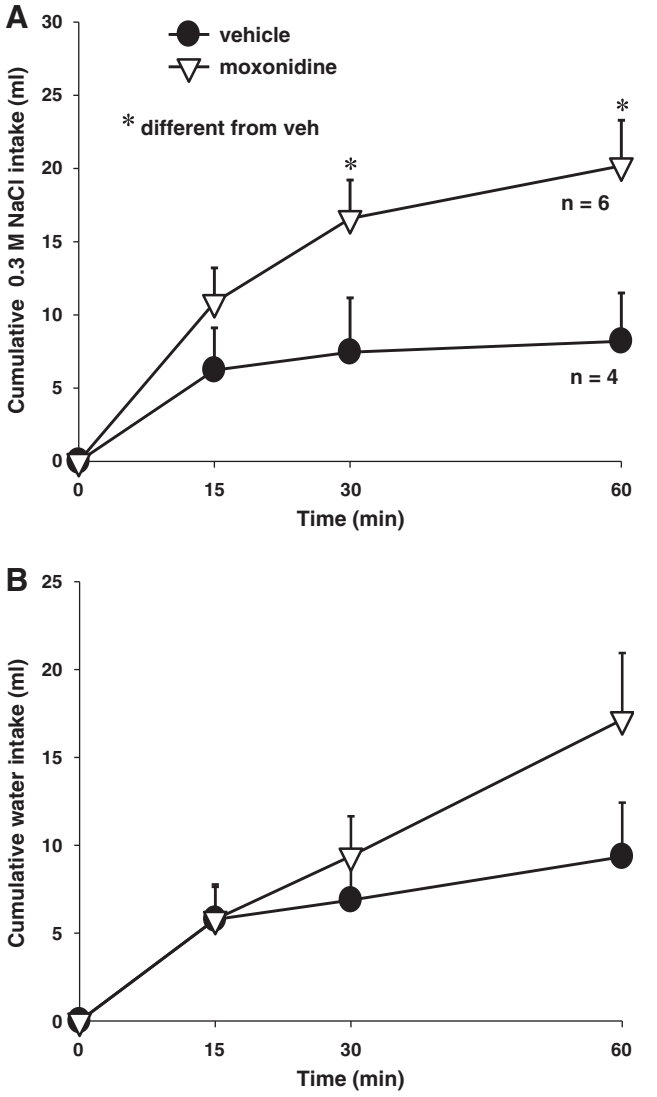

Fig. 2. Cumulative A) 0.3 M NaCl and B) water and intake by rats receiving taste reactivity tests to 0.3 M NaCl after treatment with sc furosemide + captopril combined with bilateral injections of vehicle or moxonidine (0.5 nmol/0.2 μl) into the LPBN. Results expressed as means ± SEM. n = number of animals.

NaCl [$F(1,24) = 11.9$; $p<0.05$], without significant changes in water intake [$F(1,24) = 2.2$; $p>0.05$] (Fig. 2).

In rats treated with FURO + CAP sc, moxonidine injected into the LPBN enhanced ingestive reactions at 30 and 60 min after free access to water and 0.3 M NaCl intake [$F(1,24) = 13.2$; $p<0.05$] (Fig. 3A), and decreased rejection responses at 15, 30 and 60 min after free access to water and 0.3 M NaCl intake [$F(1,24) = 20.2$; $p<0.05$] (Fig. 3B).

Most of the LPBN injections were centered in the central lateral and dorsal lateral portions of the LPBN (Fig. 4). Injections also reached the ventral lateral and external lateral portions of the LPBN, and in some rats the caudal parts of the Kölliker–Fuse nucleus. The LPBN injection sites in the present study were similar to those of previous studies that showed the effects of moxonidine on NaCl and water intake [24,33,38,39].

4. Discussion

Both LPBN vehicle and LPBN moxonidine treated animals showed enhanced ingestive responses and decreased rejection responses after FURO + CAP treatment before the access to water and NaCl solution. As compared to LPBN vehicle injections, LPBN moxonidine treatment did not affect either ingestive responses or rejection responses in sodium and water depleted rats before the rats consumed any water or 0.3 M NaCl. However, in comparison to LPBN treated vehicle controls, LPBN moxonidine treated rats continued to show enhanced ingestive reactions and reduced rejection responses even after consuming large volumes of 0.3 M NaCl and water. In other words, LPBN moxonidine treatment appears to block the satiation related declines in ingestive responses or increase in rejection responses that normally occur over the course of 0.3 M NaCl and water consumption.

The increase in ingestive and the decrease in rejection reactions to 0.3 M NaCl in FURO + CAP treated rats is consistent with previous results demonstrating that animals with experimentally-induced salt appetite show enhanced ingestive and reduced rejection oro-facial and body behaviors [40,41]. In these previous experiments where sodium depletion was induced by furosemide followed by 18 to 24 h of restricted dietary sodium, rats showed comparable increased ingestive and decreased rejection responding to IO hypertonic NaCl solution [40,41]. In the present studies Experiment 1 showed that FURO + CAP treatment, which induces a rapid onset of sodium appetite, elicits increased ingestive behaviors and decreased rejection reactions to IO hypertonic NaCl and that this occurred regardless of whether animals received LPBN vehicle or moxonidine treatment.

Interestingly the results from experiment 1 indicated that LPBN moxonidine treatment failed to enhance ingestive behaviors or decrease rejection responses to IO 0.3 M NaCl beyond those changes seen in LPBN vehicle treated rats. In light of this outcome, we considered that this outcome might reflect a ceiling effect for ingestive responses and/or a floor effect for rejection responses. Therefore, we conducted a second experiment to test taste reactivity to IO 0.3 M NaCl in FURO + CAP treated rats after fixed periods of 0.3 M NaCl and water consumption. In the second experiment rats with LPBN vehicle treatment showed a progressive reduction in ingestive responses and an increase in rejection behaviors over the course of a 1 h test period. In contrast rats with LPBN moxonidine injections maintained a high level of ingestive responses and a low level of rejection reactions to 0.3 M NaCl throughout the entire course of the 60 min test period of free access to water and sodium. In comparison to animals treated with LPBN vehicle injections, moxonidine maintained significantly increased ingestive reactions and reduced rejection responses in spite of ingesting significant amounts

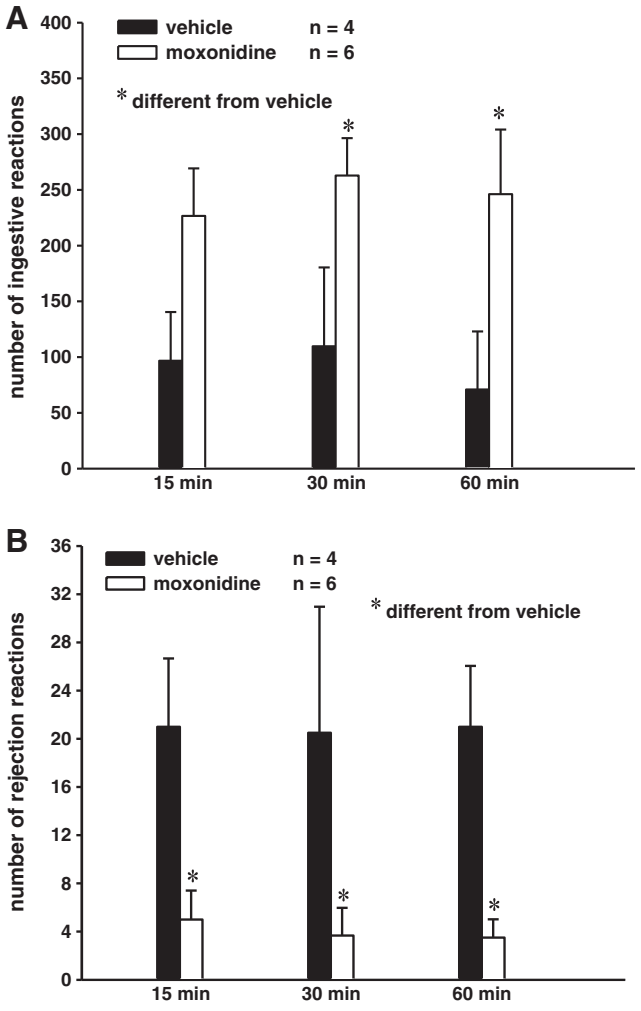

Fig. 3. A) Ingestive responses and B) rejection responses to IO infusion of 0.3 M NaCl in rats treated with sc furosemide + captopril combined with bilateral injections of vehicle or moxonidine (0.5 nmol/0.2 μl) into the LPBN at 15, 30 and 60 min after free access to 0.3 M NaCl and water. Results expressed as means ± SEM. n = number of animals.

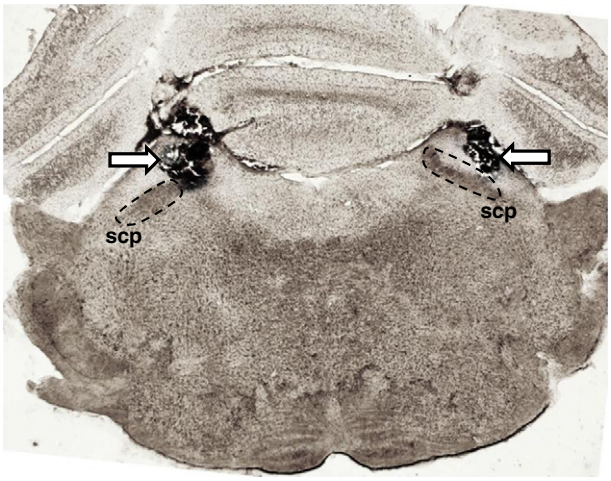

Fig. 4. Photomicrograph of a transverse brain section from an animal representative of the group tested showing the bilateral injection sites in the lateral parabrachial nuclei (arrows). scp, superior cerebellar peduncle (outlined).

of 0.3 M NaCl and water. These results suggest that LPBN moxonidine possibly reduces some type of inhibitory signals produced as a consequence of the ingestion of NaCl and water. An obvious consequence of blocking such inhibitory mechanisms would be to increase hypertonic NaCl intake in free access tests (i.e., to produce an enhanced salt appetite, as defined by increased hypertonic NaCl solution ingestion). Of course rats treated with FURO + CAP combined with LPBN moxonidine injections, stop the ingestion of hypertonic 0.3 M NaCl eventually, but this is likely to be the result of either a reduction of the action of moxonidine in the LPBN or due to stronger inhibition generated by the large quantity of NaCl and water ingested.

Previous studies have found that visceral inhibitory feedback affects NaCl intake in rats [56–60]. For example, Krause and colleagues [57] demonstrated the importance of post-ingestive signals for the satiation of salt appetite and thirst in rats treated with FURO and instrumented with gastric fistulas to allow sham drinking. These investigators found that when the fistulas were open, saline and water intakes were significantly increased as compared to that of closed fistula controls. In a similar vein, Flynn et al. [58] have found that the increased intake of hypertonic NaCl observed in spontaneously hypertensive rats (SHR) in comparison to normotensive Wistar Kyoto (WKY) rats is associated with a decrease in the decline in lick rate over the course of saline access in the SHR as compared to WKY rats. These investigators suggested that the reduced decline in lick rate evidenced by SHR may be because this strain is less responsive to ingestion-contingent inhibitory feedback [58].

Evidence indicates that interactions between taste input and inhibitory gastrointestinal, osmotic and vascular (e.g., arterial and low pressure baroreceptor) signals may act to limit excessive salt and water consumption. Information generated in the periphery is likely to influence activity at one or more central nervous system (CNS) sites where input from gustatory and various visceral sensory systems converge. The parabrachial nucleus is potentially one of these places.

Sodium taste information arrives in the CNS through the facial nerve (VII) that innervates the anterior tongue [42]. Fibers from the chorda tympani branch of the facial nerve terminate in the rostral portion of the nucleus of the solitary tract (rNTS). In turn, the rNTS sends ascending projections to the PBN. From the PBN, signals ascend to a thalamic relay, the parvocellular ventral posteromedial thalamic nucleus (VPMpc), [18,36,43]. Lesions placed in the NTS, PBN, or VPMpc disrupt the shift in taste reactivity observed in intact animals after sodium deficiency. However, only lesions in the NTS and PBN block increases in home-cage intake observed after intact animals were made sodium deficient [36]. Flynn and colleagues (36) showed that the PBN, the second synaptic relay of the ascending sodium taste information in rats, is also important for the taste reactivity to sodium in states of sodium deficit.

As sodium is consumed, neural and humoral post-ingestive signals from the gut and blood are detected by neurons in the caudal NTS and AP (see [44] for review). Viscerosensory afferent fibers in the vagus nerve are stimulated by gut distention (proportional to the volume of ingested fluid), by luminal hyperosmolarity (due to the hypertonicity of NaCl), and probably also by sodium-specific sensors within the portal vasculature as sodium is absorbed [45]. These activated vagal afferents stimulate neurons in the NTS as well as cells in the AP, some of which can directly monitor humoral variables such as plasma osmolarity.

The region of AP/mNTS also receives afferent projections from volume receptors (arterial baroreceptors and cardiopulmonary receptors), and these receptors can influence the ingestion of water and sodium [22,23,46,47]. Activation of superior vena cava–right atrial junction receptors has been shown to attenuate isoproterenol-induced water intake in intact rats, and this inhibition is abolished by electrolytic lesions of the LPBN [48]. These findings support the hypothesis that the LPBN is involved in the inhibition of drinking mediated by volume receptor stimulation. Many neurons in the caudal or medial NTS and AP project to nuclei within the LPBN [13]. In

turn, neurons in these LPBN nuclei transmit this information to a variety of limbic and hypothalamic targets in the forebrain [49,50], providing negative feedback signals that inhibit ingestive behaviors [7,10,22,23,33,38,51,52].

In summary, the present results demonstrate that moxonidine injections into the LPBN change the pattern of taste reactivity to 0.3 M NaCl by maintaining ingestive reactions, and reducing rejection responses after free access to water and 0.3 M NaCl intake. Therefore, the present results suggest that LPBN moxonidine injections reduce inhibitory signals activated as a consequence of the ingestion of hypertonic NaCl solution and water.

Acknowledgements

The authors thank Terry G. Beltz, Silas P. Barbosa, Reginaldo C. Queiroz and Silvia Fóglia for expert technical assistance, Silvana A. D. Malavolta for secretarial assistance, and Ana L. V. de Oliveira for animal care. The authors also thank Solvay Pharma and Dr. P. Ernsberger for the donation of moxonidine. This research was supported by public funding from Brazil: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP — 07/52343-8), Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG — APQ-00713-09) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES — 1419-09-9).

References

[1] Richter CP. Salt taste thresholds for normal and adrenalectomized rats. Endocrinology 1939;24:367–71.
[2] Epstein AN, Stellar E. The control of salt preference in the adrenalectomized rat. J Comp Physiol Psychol 1955;48:167–72.
[3] Morrison GR, Young JC. Taste control over sodium intake in sodium deficient rats. Physiol Behav 1972;8:29–32.
[4] Colombari DS, Menani JV, Johnson AK. Forebrain angiotensin type 1 receptors and parabrachial serotonin in the control of NaCl and water intake. Am J Physiol 1996;271:R1470–6.
[5] Edwards GL, Johnson AK. Enhanced drinking after excitotoxic lesions of the parabrachial nucleus in the rat. Am J Physiol 1991;261:R1039–44.
[6] Menani JV, Johnson AK. Lateral parabrachial serotonergic mechanisms: angiotensin-induced pressor and drinking responses. Am J Physiol 1995;269:R1044–9.
[7] Menani JV, Thunhorst RL, Johnson AK. Lateral parabrachial nucleus and serotonergic mechanisms in the control of salt appetite in rats. Am J Physiol 1996;270:R162–8.
[8] Menani JV, Colombari DSA, Beltz TG, Thunhorst RL, Johnson AK. Salt appetite: interaction of forebrain angiotensinergic and hindbrain serotonergic mechanisms. Brain Res 1998;801:29–35.
[9] Menani JV, De Luca Jr LA, Johnson AK. Lateral parabrachial nucleus serotonergic mechanisms and salt appetite induced by sodium depletion. Am J Physiol 1998;274:R555–60.
[10] Menani JV, De Luca Jr LA, Thunhorst RL, Johnson AK. Hindbrain serotonin and the rapid induction of sodium appetite. Am J Physiol 2000;279:R126–31.
[11] Ciriello J, Lawrence D, Pittman QJ. Electrophysiological identification of neurons in the parabrachial nucleus projecting directly to the hypothalamus in the rat. Brain Res 1984;322:388–92.
[12] Fulwiler CE, Saper CB. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res Rev 1984;7:229–59.
[13] Herbert H, Moga MM, Saper CB. Connections of the parabrachial nucleus to the solitary tract and the medullary reticular formation in the rat. J Comp Neurol 1990;293:540–80.
[14] Jhamandas JH, Harris KH, Petrov T, Krukoff TL. Characterization of the parabrachial nucleus input to the hypothalamic paraventricular nucleus in the rat. J Neuroendocrinol 1992;4:461–71.
[15] Jhamandas JH, Petrov T, Harris KH, Vu T, Krukoff TL. Parabrachial nucleus projection to the amygdala in the rat. Electrophysiological and anatomical observations. Brain Res Bull 1996;39:115–26.
[16] Krukoff TL, Harris KH, Jhamandas JH. Efferent projections from the parabrachial nucleus demonstrated with the anterograde tracer *Phaseolus vulgaris* leucoagglutinin. Brain Res Bull 1993;30:163–72.
[17] Lança AJ, van der Kooy D. A serotonin-containing pathway from the area postrema to the parabrachial nucleus in the rat. Neuroscience 1985;14:1117–26.
[18] Norgren R. Gustatory system. In: Paxinos G, editor. The rat nervous system. Australia: Academic Press; 1995. p. 751–71.
[19] Franchini LF, Vivas L. Distribution of Fos immunoreactivity in rat brain after sodium consumption induced by peritoneal dialysis. Am J Physiol 1999;276: R1180–7.
[20] Kobashi M, Ichikawa H, Sugimoto T, Adachi A. Response of neurons in the solitary tract nucleus, area postrema and lateral parabrachial nucleus to gastric load of hypertonic saline. Neurosci Lett 1993;158:47–50.
[21] Yamamoto T, Shimura T, Sako N, Sakai N, Tanimizu T, Wakisaka S. c-Fos expression in the parabrachial nucleus after ingestion of sodium chloride in the rat. Neuroreport 1993;4:1223–6.
[22] Johnson AK, Thunhorst RL. The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. Front Neuroendocrinol 1997;18:292–353.
[23] Johnson AK, Thunhorst RL. The neuroendocrinology, neurochemistry and molecular biology of thirst and salt appetite. In: Lajtha A, Laustein JD, editors. Handbook of Neurochemistry and Molecular Neurobiology: Behavioral Neurochemistry, Neuroendocrinology and Molecular Neurobiology. 3rd Edition. New York: Springer; 2007. p. 641–87.
[24] Andrade-Franzé GMF, Andrade CAF, De Luca Jr LA, De Paula PM, Menani JV. Lateral parabrachial nucleus and central amygdala in the control of sodium intake. Neuroscience 2010;165:633–41.
[25] Andrade-Franzé GMF, Andrade CAF, De Luca Jr LA, De Paula PM, Colombari DSA, Menani JV. Lesions in the central amygdala impair sodium intake induced by the blockade of the lateral parabrachial nucleus. Brain Res 2010;1332:57–64.
[26] De Castro e Silva E, Fregoneze JB, Johnson AK. Corticotropin-releasing hormone in the lateral parabrachial nucleus inhibits sodium appetite in rats. Am J Physiol 2005;290:R1136–41.
[27] De Gobbi JIF, Beltz TG, Johnson RF, Menani JV, Thunhorst RL, Johnson AK. Non-NMDA receptors in the lateral parabrachial nucleus modulate sodium appetite. Brain Res 2009;1301:44–51.
[28] De Gobbi JIF, De Luca Jr LA, Menani JV. Serotonergic mechanisms of the lateral parabrachial nucleus on DOCA-induced sodium intake. Brain Res 2000;880:131–8.
[29] De Gobbi JIF, Menani JV, Beltz TG, Johnson RF, Thunhorst RL, Johnson AK. Right atrial stretch alters fore- and hind-brain expression of c-fos and inhibits the rapid onset of salt appetite. J Physiol 2008;586:3719–29.
[30] De Oliveira LB, Callera JC, De Luca Jr LA, Colombari DSA, Menani JV. GABAergic mechanisms of the lateral parabrachial nucleus on sodium appetite. Brain Res Bull 2007;73:238–47.
[31] De Oliveira LB, De Luca Jr LA, Colombari DSA, Colombari E, Menani JV. Opioid activation in the lateral parabrachial nucleus induces hypertonic sodium intake. Neuroscience 2008;155:350–8.
[32] Gasparini S, De Luca Jr LA, Colombari DS, De Paula PM, Barbosa SP, Menani JV. Adrenergic mechanisms of the Kölliker-Fuse/A7 area on the control of water and sodium intake. Neuroscience 2009;164(2):370–9.
[33] Andrade CAF, Barbosa SP, De Luca Jr LA, Menani JV. Activation of alpha2-adrenergic receptors into the lateral parabrachial nucleus enhances NaCl intake in rats. Neuroscience 2004;129:25–34.
[34] Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 1978;143:263–79.
[35] Grill HJ, Berridge KC. Taste reactivity as a measure of the neural control of palatability. In: Sprague JM, Epstein AN, editors. Progress in Psychobiology and Physiological Psychology. Orlando: Academic Press; 1985. p. 1–61.
[36] Flynn FW, Grill HJ, Schulkin J, Norgren R. Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviors. Behav Neurosci 1991;105:944–54.
[37] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 5th ed. San Diego, CA: Academic Press; 2004.
[38] Andrade CAF, De Luca Jr LA, Colombari DSA, Menani JV. Alpha2-adrenergic activation in the lateral parabrachial nucleus induces NaCl intake under conditions of systemic hyperosmolarity. Neuroscience 2006;142:21–8.
[39] Andrade CAF, De Luca Jr LA, Colombari DSA, Menani JV. Enhancement of meal-associated hypertonic NaCl intake by monoxidine into the lateral parabrachial nucleus. Behav Brain Res 2007;183:156–60.
[40] Berridge KC, Flynn FW, Schulkin J, Grill HJ. Sodium depletion enhances salt palatability in rats. Behav Neurosci 1984;98:652–60.
[41] Grill HJ, Bernstein IL. Strain differences in taste reactivity to NaCl. Am J Physiol 1988;255:R424–30.
[42] Frank ME, Contreras RJ, Hettinger TP. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. J Neurophysiol 1983;50:941–60.
[43] Hamilton RB, Norgren R. Central projections of gustatory nerves in the rat. J Comp Neurol 1984;222:560–77.
[44] Geerling JC, Loewy AD. Sodium deprivation and salt intake activate separate neuronal subpopulations in the nucleus of the solitary tract and the parabrachial complex. J Comp Neurol 2007;504(4):379–403.
[45] Morita H, Yamashita Y, Nishida Y, Tokuda M, Hatase O, Hosomi H. Fos induction in rat brain neurons after stimulation of the hepatoportal Na-sensitive mechanism. Am J Physiol 1997;272:R913–23.
[46] Norgren R. The central organization of the gustatory and visceral systems in the nucleus of the solitary tract. In: Katsuki Y, Norgren R, Sato M, editors. Brain mechanisms of sensation. New York: Wiley; 1981. p. 143–60.
[47] Johnson AK. The sensory psychobiology of thirst and salt appetite. Med Sci Sports Exerc 2007;39(8):1388–400.
[48] Ohman LE, Johnson AK. Role of lateral parabrachial nucleus in the inhibition of water intake produced by right atrial stretch. Brain Res 1995;695:275–8.
[49] Bernard JF, Alden M, Besson JM. The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: a *Phaseolus vulgaris* leucoagglutinin (PHA-L) study in the rat. J Comp Neurol 1993;329: 201–29.
[50] Bester H, Besson JM, Bernard JF. Organization of efferent projections from the parabrachial area to the hypothalamus: a *Phaseolus vulgaris*-leucoagglutinin study in the rat. J Comp Neurol 1997;383:245–81.
[51] Contreras RJ, Stetson PW. Changes in salt intake lesions of the area postrema and the nucleus of the solitary tract in rats. Brain Res 1981;211:355–66.

[52] Menani JV, Johnson AK. Cholecystokinin actions in the parabrachial nucleus: effects on thirst and salt appetite. Am J Physiol 1998;275:R1431–7.
[53] Peciña S, Berridge KC. Brainstem mediates diazepam enhancement of palatability and feeding: microinjections into fourth ventricle versus lateral ventricle. Brain Res 1996;727(1–2):22–30.
[54] Peciña S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci 2005;25(50): 11777–86.
[55] Grill HJ, Spector AC, Schwartz GJ, Kaplan JM, Flynn FW. Evaluating taste effects on ingestive behavior. In: Toates FM, Rowland NE, editors. Feeding and drinking. Amsterdam: Elsevier; 1987. p. 151–88.
[56] Flynn FW, Smith M, Bieber S. Differential effects of intraventricular injections of tachykinin NK1 and NK3 agonists on normal and sham drinking of NaCl by sodium deficient rats. Behav Neurosci 1999;113:776–86.
[57] Krause EG, de Kloet AD, Sakai RR. Post-ingestive signals and satiation of water and sodium intake of male rats. Physiol Behav Apr 19 2010;99(5):657–62.
[58] Flynn FW, Culver B, Newton SV. Salt intake by normotensive and spontaneously hypertensive rats: two-bottle and lick rate analyses. Physiol Behav Apr 2003;78(4–5): 689–96.
[59] Tordoff MG, Schulkin J, Freidman MI. Further evidence for hepatic control of salt intake. Am J Physiol Regul Integr Comp Physiol 1987;253:R444–9.
[60] Wolf G, Schulkin J, Simson PE. Multiple factors in the satiation of salt appetite. Behav Neurosci 1984;98:661–73.